Chapter 6

Hypothermic Oxygenated Machine Perfusion Reduces Bile Duct Reperfusion Injury after Transplantation of Donation after Circulatory Death Livers

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ABSTRACT

Introduction: Dual hypothermic oxygenated machine perfusion (DHOPE) of the liver has been advocated as a method to reduce ischemia-reperfusion injury. This study aimed to determine whether DHOPE reduces IR injury of the bile ducts in DCD liver transplantation.

Methods: In a recently performed phase 1-trial, ten DCD livers were preserved with DHOPE after static cold storage (SCS) (www.trialregister.nl NTR4493). Bile duct biopsies were obtained at the end of SCS (before DHOPE; baseline) and after graft reperfusion in the recipient. Histological severity of biliary injury was graded according to an established semi-quantitative grading system. Twenty liver transplantations using DCD livers not preserved with DHOPE served as control.

Results: Baseline characteristics and the degree of bile duct injury at baseline (end of SCS) were similar between both groups. In controls, degree of stroma necrosis ($p = 0.002$) and injury of the deep peribiliary glands ($p = 0.02$) increased after reperfusion, compared to baseline. In contrast, in DHOPE preserved livers the degree of bile duct injury did not increase after reperfusion. Moreover, there was less injury of deep peribiliary glands ($p = 0.04$) after reperfusion in the DHOPE group, compared to controls.

Conclusion: This study suggests that DHOPE reduces ischemia-reperfusion injury of bile ducts after DCD liver transplantation.

INTRODUCTION

The worldwide shortage of donor livers for transplantation has led to efforts to increase the number of available grafts. In countries such as the Netherlands, Spain and the UK, this has led to a more frequent use of donation after circulatory death (DCD) livers for transplantation.[1,2] Unfortunately, DCD liver grafts have a 3-fold higher risk of developing non-anastomotic biliary strictures (NAS) after transplantation, compared to donation after brain death (DBD) liver grafts. The reported incidence of NAS ranges between 16-31% in DCD versus 3-13% in DBD liver grafts.[3-5] This type of biliary complication is regarded as a major complication after DCD liver transplantation as it often requires multiple endoscopic interventions and leads to re-transplantation in 16% of patients and death in 6%.[6,7]

Although the etiology of NAS is not fully understood, the duration of cold and warm ischemia during transplantation has been recognized as a major risk factor for NAS.[8-10] Ischemic conditions lead to a complex cascade of events resulting in ischemia-reperfusion (IR) injury.[11] Three independent clinical studies recently demonstrated that the majority of donor livers have histological evidence of extensive biliary IR injury at the time of transplantation.[12-14] Especially the degree of biliary epithelial loss, mural necrosis, and injury of the deep peribiliary glands (PBG) and peribiliary vascular plexus (PVP) at the time of transplantation has been associated with the development of NAS after transplantation.[12-14] The PBG contain biliary stem/progenitor cells that are involved in the regeneration and repair of the bile duct epithelium after severe injury.[15-17] Therefore, it has been hypothesized that reduced regenerative capacity of the bile ducts due to damage of the PBG plays an important role in the development of NAS after transplantation.[14,18]

A short period of hypothermic machine perfusion (HMP) after conventional static cold storage (SCS) has been shown to reduce IR injury of donor livers.[19,20] End-ischemic HMP results in a reduction of hepatocyte apoptosis and necrosis, mitochondrial and nuclear injury, endothelial injury, Kupffer cell activation, and the subsequent host immune response.[19-23] Furthermore, animal studies have suggested that HMP reduces IR injury of the bile ducts, as indicated by an improved biliary epithelial cell function, reduced biliary injury markers, and less histological bile duct wall necrosis, epithelial cell loss, and arteriolonecrosis of the PVP, compared to SCS alone.[24-26] The first clinical series of end-ischemic HMP have demonstrated that this method is safe and may lead to a lower incidence of biliary complications after transplantation.[27-31] However, formal proof for such a protective effect of HMP should come from prospective randomized trials that are currently ongoing (ClinicalTrials.gov NCT02584283).

Despite the recognized beneficial effect of HMP on IR injury and a suggested reduced risk of NAS after transplantation, there are no studies that have examined the effect of HMP on IR injury of human bile ducts. In the present study, we aimed to determine whether dual oxygenated HMP (DHOPE) reduces reperfusion injury of the bile ducts in DCD liver transplantation, by performing a systematic histological comparison of donor bile ducts before and after graft reperfusion.

MATERIALS AND METHODS

Study population

A recently performed phase 1 study in our center included ten consecutive patients who underwent DCD liver transplantation between April and November 2014.[31] The donor livers were preserved with DHOPE for two hours after conventional SCS. Informed consent for DHOPE was obtained. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Medical Ethics Committee of the UMCG (approval number METc2014.100).

A control group consisted of patients who underwent DCD liver transplantation in our center between February 2012 and September 2015. During this period, bile duct biopsies were routinely obtained when the bile duct was long enough. All patients in whom bile duct biopsies were obtained, were included in the control group without application of exclusion criteria.

Study protocol

All DCD livers were procured by one of the regional multi-organ recovery teams using a rapid procurement protocol with aortic cold flush-out and additional portal vein flush on the back table, followed by SCS. Upon arrival in our center, the liver was inspected and prepared for perfusion by placement of cannulas in the portal vein and aorta. The cannulas were connected to the disposable tubing set of the Liver Assist device (Organ Assist, Groningen, The Netherlands). The livers were perfused for at least two hours with 4 L of UW Machine Perfusion Solution (Bridge-to-Life Ltd, Norfolk, UK) at a temperature of 10-12 °C. Two rotary pumps enabled pressure-controlled perfusion with pulsatile mean arterial pressure of 25 mmHg and a continuous portal pressure of 5 mmHg. The perfusion solution was oxygenated by two hollow fiber membrane oxygenators with 100% FiO2 at 500 ml/min per oxygenator.

Bile duct biopsies

Bile duct biopsies were obtained from the common bile duct, as described previously.[14] The biopsies were at the end of SCS (baseline) and after graft reperfusion in the recipient, shortly before constructing the bile duct anastomosis. Baseline biopsies were collected after SCS during the back table procedure, which was followed by DHOPE. All samples were fixed in 4% formalin, subsequently embedded in paraffin and 4µm thick slides were stained with hematoxylin & eosin (H&E). The slides were examined using light microscopy and scanned with a Hamamatsu device.

Histological grading of biliary injury

Biliary injury was graded according to an established semi-quantitative histological grading system described by Hansen et al. and modified by Op den Dries et al.[13,14] (Table 1). Biopsies were scored without knowledge of the clinical data by two investigators separately (RvR and OBvL) under supervision of two dedicated liver pathologists (ASHG and MvdH). In case of discordant results, slides were examined by a third investigator.

Table 1. Histological grading of bile duct injury

Item	Histological Characteristic	Grading
1. Biliary epithelium loss	*Absence of epithelial cells lining the bile duct lumen*	0: no loss 1: ≤50% of the bile duct with absent epithelial lining 2: >50%
2. Mural stroma necrosis	*Necrosis of the bile duct wall*	0: no necrosis 1: ≤25% of the wall necrotic 2: >25% and ≤50% 3: >50% and ≤75% 4: >75%
3. Peribiliary vascular plexus damage	*Damage to the vessels such as subendothelial edema*	0: no vascular lesions 1: ≤50% of the vessels damaged 2: >50%
4. Arteriolonecrosis	*Loss of endothelial nuclei of arterioles and media degeneration*	0: no arteriolonecrosis 1: ≤50% of the arteries necrotic 2: >50%
5. Thrombosis	*Presence of micro-thrombi*	0: no microthrombi 1: microthrombi present
6. Intramural bleeding	*Presence of erythrocytes in the bile duct wall*	0: no bleeding 1: ≤50% of the bile duct wall 2: >50%
7. Periluminal PBG loss	*Absence of epithelial cells in the PBG close to the lumen*	0: no loss 1: ≤50% loss 2: >50% loss
8. Deep PBG loss	*Absence of epithelium in the PBG located deep in the bile duct wall*	0: no loss 1: ≤50% loss 2: >50% loss

Abbreviations: PBG; peribiliary glands.

Statistical analyzes

Continuous variables were presented as median (interquartile range) or mean (standard deviation) when appropriate and were compared between groups with the 2-tailed Mann-Whitney test. Categorical variables were presented as number (percentage) and compared between groups with the Pearson chi-square test or the Fisher's exact test where appropriate. P values < 0.05 were defined as significant. Statistical analyses were performed using SPSS version 22.0 for Windows.

RESULTS

Donor and recipient characteristics

Between April and November 2014, ten patients were included in the DHOPE phase 1 trial. Detailed clinical results have been reported previously.[31] The control group consisted of 20 patients in whom bile duct biopsies were obtained, out of a total of 69 patients who underwent a DCD liver transplantation in our center between February 2012 and September 2015. There were no differences in donor and recipient characteristics between the 20 control patients and the remaining 49 recipients of a DCD graft (data not shown). Baseline clinical characteristics of the DHOPE group and the control group are summarized in Table 2. The Eurotransplant donor risk index (ET-DRI) was similar in the DHOPE and the control group. The donors in the DHOPE group had a higher latest

Table 2. Donor and recipient characteristics

Characteristic	DHOPE group (n=10)	Control group (n=20)	P value
Donor characteristics			
Eurotransplant donor risk index*	2.30 (1.81-2.53)	2.22 (1.67-2.54)	0.98
Age (years)	53 (47-57)	49 (34-55)	0.18
BMI (kg/m²)	23.0 (19.9-24.1)	23.6 (22.0-26.0)	0.25
Latest ALT (U/L)	72 (39-125)	29 (19-46)	0.008
Peak ALT (U/L)	121 (42-271)	33 (20-46)	0.004
Latest GGT (U/L)	50 (19-102)	39 (17-70)	0.75
Preservation characteristics			
Preservation fluid (UW vs. HTK)	10 (100%)	18 (90%)	0.54
Asystole time (min)#	15 (13-17)	15 (12-19)	0.95
Donor warm ischemia time (min)^	26 (23-42)	33 (29-41)	0.35
Cold ischemia time (min)**	358 (314-398)	426 (402-485)	0.002
Total preservation time (min)##	521 (469-592)	430 (407-485)	0.002
Anastomosis time (min)^^	34 (30-49)	33 (31-43)	0.88
Recipient characteristics			
Age (years)	57 (54-62)	55 (47-63)	0.50
Sex (male)	6 (60%)	9 (45%)	> 0.99
MELD score***	16 (15-22)	20 (13-24)	0.56
Underlying disease:			0.08
Alcoholic cirrhosis	3 (30%)	3 (15%)	
NASH	5 (50%)	3 (15%)	
Primary sclerosing cholangitis	1 (10%)	4 (20%)	
Primary biliary cirrhosis	0	0	
Autoimmune hepatitis	0	3 (15%)	
Hepatitis B or C	1 (10%)	1 (5%)	
Hepatocellular carcinoma	0	0	
Cryptogenic	0	3 (15%)	
Other	0	4 (20%)	

** Eurotransplant donor risk index is a validated tool to assess the risk of liver graft failure[37]. # Asystole time was defined as time between circulatory arrest and in situ aortic cold flush. ^ Donor warm ischaemia time was defined as the time interval between withdrawal of donor life support and initiation of in situ aortic cold flush. ** Cold ischemia time was defined as the interval between start aortic cold flush and end of static cold storage excluding the duration of DHOPE. ## Total preservation time was defined as the interval between start aortic cold flush in the donor and portal reperfusion in the recipient. ^^ Anastomosis time was defined as the interval between donor liver out of ice and revascularization. *** MELD score was defined as the highest of laboratory derived MELD score or the (non) standard exception MELD score.*

Abbreviations: ALT, alanine aminotransferase; DCD, donation after circulatory death; GGT, gamma-glutamyl transferase; DHOPE, dual hypothermic oxygenated machine perfusion; HTK, histidine-tryptophan-ketoglutarate; MELD, model for end stage liver disease; NASH, non-alcoholic steatohepatitis; UW, University of Wisconson.

serum alanine aminotransferase (ALT) and peak serum ALT concentration, compared to the donors in the control group: latest ALT 72 U/L (39-125 U/L) vs. 29 U/L (19-46 U/L), respectively ($p = 0.008$); peak ALT 121 U/L (42-271 U/L) vs. 33 U/L (20-46 U/L), respectively ($p < 0.001$). The cold ischemia time was shorter in the DHOPE group, but the total preservation time in the DHOPE group was longer than in the control group: 521 min (469-592 min) vs. 430 min (407-485 min), respectively ($p = 0.002$).

Histological evidence of bile duct injury

The results of bile duct injury scorings based on the semi-quantitative histological grading system are summarized in Table 3. There were no discordant results between the two investigators. As expected, at baseline there were no significant differences between the two groups for any item of the histological grading system (Figure 1). In the control group, the histological bile duct damage after reperfusion was more severe than at baseline. Especially the degree of mural stroma necrosis and the degree of deep PBG injury increased after reperfusion (p = 0.002 and p = 0.02, respectively). In contrast to the control group, there was no increase in the severity of histological biliary injury after reperfusion in the DHOPE group (Table 3).

Table 3. Comparison of histological bile duct injury

Bile duct wall component	DHOPE group			Control group		
	Baseline	After reperfusion	P value	Baseline	After reperfusion	P value
Biliary epithelium loss			> 0.99			-
Grade 0	-	-		-	-	
Grade 1	10%	14%		-	-	
Grade 2	90%	86%		100%	100%	
Mural stroma necrosis			0.25			0.002
Grade 0	90%	57%		50%	9%	
Grade 1	-	-		43%	9%	
Grade 2	10%	29%		-	36%	
Grade 3	-	14%		7%	46%	
Peribiliary vascular plexus damage			0.38			0.72
Grade 0	50%	57%		43%	36%	
Grade 1	50%	29%		43%	36%	
Grade 2	-	14%		14%	28%	
Arteriolonecrosis			> 0.99			0.57
Grade 0	90%	100%		93%	82%	
Grade 1	10%	-		-	-	
Grade 2	-	-		7%	18%	
Thrombosis			> 0.99			> 0.99
Grade 0	90%	100%		93%	91%	
Grade 1	10%	-		7%	9%	
Intramural bleeding			-			0.11
Grade 0	100%	100%		100%	73%	
Grade 1	-	-		-	18%	
Grade 2	-	-		-	9%	
Periluminal PBG loss			0.24			0.23
Grade 0	-	14%		-	-	
Grade 1	30%	29%		21%	-	
Grade 2	70%	57%		79%	100%	
Deep PBG loss			0.64			0.02
Grade 0	40%	43%		36%	-	
Grade 1	40%	43%		64%	73%	
Grade 2	20%	14%		-	27%	

Abbreviations: DHOPE, dual hypothermic oxygenated machine perfusion; PBG, peribiliary glands; SCS, static cold storage.

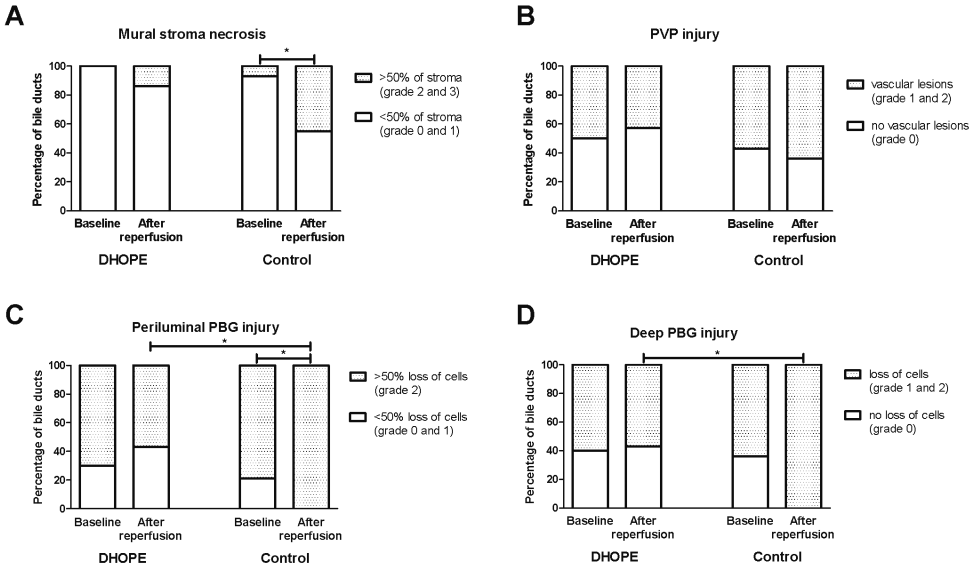

Figure 1. Degree of injury of the bile ducts of DCD livers treated with DHOPE versus controls after static cold storage and after reperfusion in the recipient. *(A) The degree of mural stroma necrosis increased after reperfusion compared to baseline in the control group (p < 0.001), but not in the DHOPE group. (B) No differences were observed for the degree of injury of the peribiliary vascular plexus. (C) The periluminal PBG of livers treated with DHOPE demonstrated less injury after reperfusion than in the control group (p = 0.04). Additionally, the injury of the deep PBG in the control group increased after reperfusion compared to baseline (p = 0.02). (D) The deep PBG in the livers treated with DHOPE demonstrated less damage after reperfusion than in the control group (p = 0.04). Asterisks indicated a P value < 0.05. Abbreviations: DCD, donation after circulatory death; DHOPE, dual hypothermic oxygenated machine perfusion; PBG, peribiliary glands; PVP, peribiliary vascular plexus.*

When comparing the severity of post-reperfusion bile duct injury between the two study groups, livers in the DHOPE group displayed significantly less injury and loss of cells in the periluminal (p = 0.04) and the deep PBG (p = 0.04), compared to controls (Figures 1-3). No differences were observed in the severity of arteriolonecrosis, stroma necrosis, or injury of the PVP between the two groups after reperfusion.

Clinical outcomes

The overall clinical outcome after transplantation of the patients in the DHOPE group has been described in detail previously.[31] Complete one-year follow up was available in all patients. The incidence of NAS within one year after transplantation was 10% in the DHOPE group and 35% in the control group (p = 0.15). NAS was defined as bile duct stenosis at any location in the biliary tree (intra- or extrahepatic, but not at the site of the anastomosis) as detected by endoscopic retrograde or magnetic resonance cholangiography, with cholestatic manifestations such as jaundice, cholangitis, or elevated laboratory tests, and in the presence of a patent hepatic artery. One recipient of a DHOPE preserved liver developed local NAS in segment 2 and 3 of the liver 5

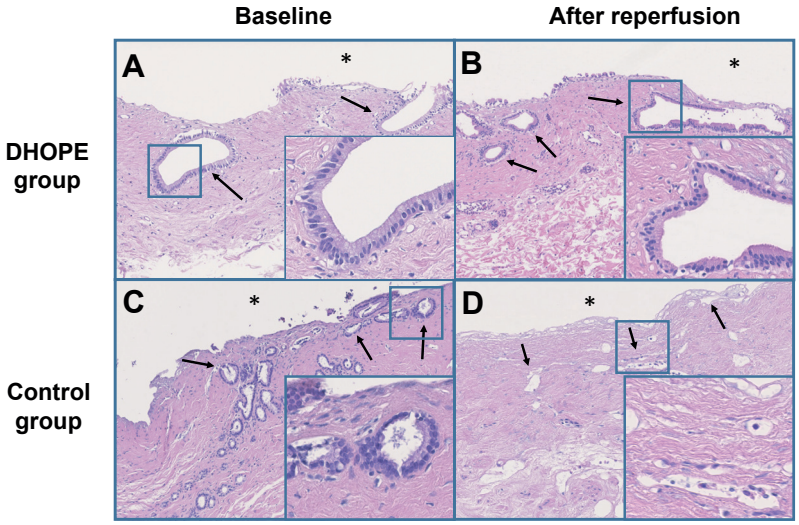

Figure 2. Representative histologic examples of periluminal PBG in the common bile duct. *(A) Bile duct at baseline of a DCD liver in the DHOPE group. (B) Bile duct after reperfusion of a DCD liver in the DHOPE group. (C) Bile duct at baseline of a DCD liver in the control group. (D) Bile duct after reperfusion of a DCD liver in the control group. The insert represents a higher magnification of the periluminal PBG (400x). Bile ducts of livers preserved with DHOPE displayed significantly less epithelial cell loss of the periluminal PBG, compared to control livers. Original magnification was 200x. Arrows indicate periluminal PBG. Asterisks indicate lumen of the bile duct. Abbreviations: DCD, donation after circulatory death; DHOPE, dual hypothermic oxygenated machine perfusion; PBG, peribiliary glands.*

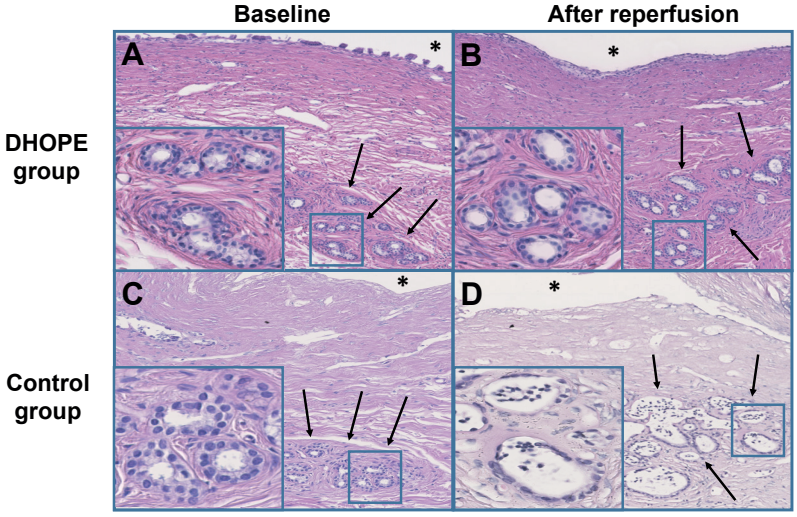

Figure 3. Representative histologic examples of deep PBG in the common bile duct of DCD liver grafts. *(A) Bile duct at baseline of a DCD liver in the DHOPE group. (B) Bile duct after reperfusion of a DCD liver in the DHOPE group. (C) Bile duct at baseline of a DCD liver in the control group. (D) Bile duct after reperfusion of a DCD liver in the control group. The insert represents a higher magnification of the deep PBG (400x). Arrows indicate deep PBG. Asterisks indicate the lumen of the bile duct. Bile ducts of livers preserved with DHOPE displayed significantly less epithelial cell loss of the deep PBG, compared to control livers. Original magnification was 200x. Abbreviations: DCD, donation after circulatory death; DHOPE, dual hypothermic oxygenated machine perfusion; PBG, peribiliary glands.*

months after transplantation, which was successfully treated with endoscopic stenting. None of the patients in the DHOPE group required re-transplantation for NAS. In contrast, 4 (20%) in the control group required re-transplantation for NAS at a median of 9 (6-12) months after transplantation.

DISCUSSION

In the current study, we aimed to determine whether end-ischemic oxygenated hypothermic machine perfusion (DHOPE) of human DCD liver grafts reduces IR injury of the bile ducts after transplantation. The results of this study clearly demonstrated a reduction in biliary IR injury of DHOPE-preserved DCD livers, compared to DCD livers that did not undergo DHOPE. These findings provide important new insight in the protective mechanism of end-ischemic DHOPE and are in line with the clinically observed reduction in the incidence of NAS after DCD liver transplantation when DHOPE is applied.[27-31]

In accordance with previous reports[12,14], all DCD livers included in this study displayed histological signs of substantial bile duct injury at the end of SCS. As expected, the degree of bile duct injury after SCS (baseline) was not different between livers that underwent DHOPE or not. In control livers that were not treated with DHOPE, the degree of biliary injury worsened after graft reperfusion in the recipient, a phenomenon that was not observed in DHOPE preserved livers. Especially, the severity of mural stroma necrosis and injury of the deep peribiliary glands increased after reperfusion of the control livers, but not of the DHOPE preserved livers. When comparing post-reperfusion biopsies of bile ducts of DHOPE-preserved livers and controls, significantly less histological injury of both the periluminal and deep PDG was found in the DHOPE group.

In a large clinical study including 128 liver transplant recipients we have previously shown that the severity of bile duct injury at the end of SCS is a strong risk factor for the development of NAS after transplantation.[14] Upon reperfusion of a liver graft in the recipient, the degree of biliary injury increases further and this may result in irreversible damage of essential components of the bile duct wall, such as the mural stroma, peribiliary vasculature, and PBG.[12,14] Obviously, end-ischemic DHOPE cannot have a protective effect on biliary injury that is already present after SCS. However, the current study indicates that DHOPE does prevent further worsening of bile duct injury after graft reperfusion.

It is well known that the majority of tissue damage due to IR injury occurs in the reperfusion period after restoration of blood flow to the graft.[11] Some of the key factors in IR injury are the depletion of cellular energy content (especially intracellular adenosine triphosphate (ATP) and the formation of reactive oxygen species (ROS) due to mitochondrial dysfunction.[11,32] While ROS formation results in oxidative damage of cellular structures, such as cell membranes and nuclear DNA, depletion and insufficient restoration of ATP results in cell death due to insufficient metabolic function. Although the conditions for IR injury are generated during cold ischemic preservation, the sequelae of events that lead to the full blown IR injury is not activated until after warm reperfusion.[11] Experimental and clinical studies have demonstrated that one of the key protective mechanisms

of hypothermic oxygenated machine perfusion is a full restoration of cellular ATP content and "resuscitation" of mitochondria, resulting in a significant reduction of ROS production after subsequent warm graft reperfusion in the recipient.[21,22,33,34] In addition, and downstream of this, oxygenated HMP results in a reduction of Kupffer cell activation and less secondary activation of the innate immune system.[21,22,33,34] Altogether, these protective effects have been shown to result in a reduction of hepatocellular IR injury. Our data on the reduction of biliary IR injury are in line with these previous studies and demonstrate that not only hepatocellular IR injury, but also IR injury of the bile ducts is attenuated by oxygenated HMP.

The DHOPE-preserved livers in the present study demonstrated significantly less severe injury of the deep and periluminal PBG after reperfusion, compared to the control livers that did not undergo DHOPE. This histological finding is clinically relevant since the PBGs have been identified as a local niche of biliary progenitor cells that contribute to the regeneration of biliary epithelium after injury.[15-17,35] Severe injury of the deep PBG at the time of transplantation is a significant risk factor for the development of NAS.[14] Therefore, the protective effect of DHOPE on PBG may lead to better preserved regenerative biliary capacity followed by a reduction of the incidence of NAS. This is supported by the (non-significant) low incidence of NAS observed after transplantation of DCD livers preserved with HMP in the first clinical series.[27-31] However, formal evidence that oxygenated HMP reduces the incidence of NAS should come from an adequately powered randomized, controlled trial. Such a multicenter trial has recently been initiated in transplant centers in the Netherlands, Belgium and United Kingdom (ClinicalTrials.gov NCT02584283).

Bile duct biopsies in this study were obtained from the extrahepatic bile ducts, while NAS mainly occurs in the intrahepatic bile ducts. However, previous studies have shown that the degree of injury of the extrahepatic bile duct correlates well with the degree of injury of the intrahepatic bile ducts[36] and histological assessment of the extrahepatic bile duct can predict the development of NAS.[14]

In contrast to a previous study on DHOPE using a porcine DCD liver model, we did not find a reduction in the degree of injury of the PVP and arteriolonecrosis in the human liver bile ducts after DHOPE.[24] In fact, in the current study we did not find a significant increase in the degree of PVP injury and arteriolonecrosis after reperfusion, compared to baseline in both groups. In the current study the post-reperfusion bile duct biopsy was taken 1-2 hours after graft reperfusion and this time interval may have been too short for histologically detectable vascular injury to develop.

Limitations of this study are the sample size and the use of historical controls. The controls used in this histological study were slightly different from the ones used in our previous report on clinical outcome after transplantation of DHOPE-preserved DCD livers[31], because the control group consisted of all available paraffin embedded bile duct biopsies from DCD liver grafts without application of exclusion criteria. Although most baseline characteristics such as ET-DRI were similar between the two study groups, the donors in the DHOPE group had significantly higher serum ALT levels, compared to donors in the control group. As ALT is a marker for hepatocellular injury, the DHOPE group consisted of livers with slightly more pre-existing injury than the control group.

Therefore, the observed beneficial effects of DHOPE might have been more pronounced if the ALT levels would have been equivalent between the groups. As expected, the preservation method DHOPE affected the length of preservation periods in the study groups: the total preservation time was longer but the cold ischemia time was shorter in the DHOPE group compared to the control group. In the intervention group the donor liver is machine perfused while the recipient surgery is performed. In the control group, the donor liver remains in the ice box during this period. The shorter cold ischemia time in the DHOPE group may have caused an advantage, while the longer total preservation time may have caused a disadvantage. However, the median difference in cold ischemia time was only 30 minutes and baseline levels of bile duct injury were similar between the groups.

In conclusion, this study demonstrates that DHOPE attenuates IR injury of the bile ducts after transplantation of DCD liver grafts. In particular, DHOPE contributed to a better preservation of the PBG and, as such, may preserve the regenerative capacity of the donor bile ducts leading to a reduced risk of biliary complications after transplantation.

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